EFFECTS OF CLINOSTAT ROTATION ON AURELIA STATOLITH SYNTHESIS

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Aurelia ephyrae develop eight graviceptors (rhopalia) during their metamorphosis from polyps, which are used for positional orientation with respect to gravity.

In three experiments for each speed of 1/15, 1/8, 1/4, 1/2, 1, and 24 rpm, groups of six polyps were rotated in the horizontal or vertical plane (control) using clinostats. Other controls were kept stationary in the two planes. Ten ephyrae from each group were collected after 5-6 days at 27°C in iodine and the number of statoliths per rhopalium were counted. Statistical analyses of statolith numbers revealed that horizontal clinostat rotation at 1/4 and 1/2 rpm caused the formation of significantly fewer statoliths per rhopalium than were found in controls. The finding that these slow rates of rotation reduces statolith numbers suggests that the developing ephyrae were disoriented with respect to gravity at these speeds, causing fewer statocytes to differentiate or to mineralize.

INTRODUCTION

Using the Aurelia Metamorphosis Test System (Spangenberg, 1984), we have been investigating the effects of clinostat rotation in the horizontal plane on the development of ephyrae and the synthesis of their statoliths.

Aurelia polyps are especially suited for gravity-related research because they are very small (2-4 mm), form ephyrae with gravity sensing structures (rhopalia) in 5-6 days, and can be used for clinostat studies. During iodine-induced metamorphosis (Spangenberg, 1967), ephyrae develop in sequential order from the oral to the aboral end of the polyps. Eight rhopalia with sacs of statoliths at their distal ends form per ephyra. These statoliths are composed of calcium sulfate dihydrate (Spangenberg and Beck, 1968) and only one statolith forms per statocyte.

METHODS

Two clinostats, made according to the design of Tremor and Souza (1972), were used for these studies. Jellyfish polyps were impaled head downwards on cactus spines embedded in paraffin in the conical bottoms of autoanalyzer capsules (Figure 1). The capsules were filled with 10^{-5} M iodine in artificial sea water (ASW) prepared according to Spangenberg (1967). The caps of the capsules were filled with paraffin to eliminate bubble formation in the capsules, and the cap-capsule junction was covered with pressure sensitive tape to prevent leakage of the solution or evaporation. The capsules were tightly held in a 9 inch long glass tube with plastic joiners and the tube was attached to the shaft of the clinostat.

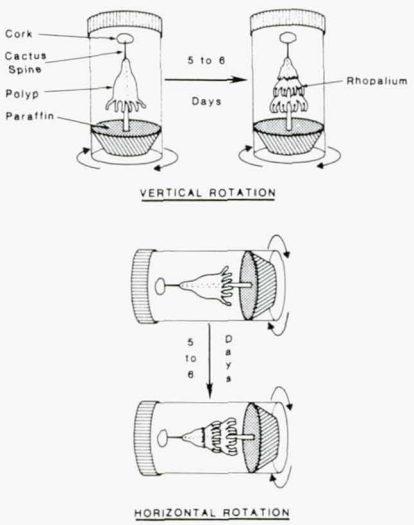

Figure 1. Orientation of the polyps and developing ephyrae during clinostat rotation.

Three tests were done for each clinostat speed using groups of 6 polyps (one per capsule) and speeds of 1/15, 1/8, 1/4, 1/2, 1, and 24 rpm were used. For each test, polyps were (1) rotated in a horizontal plane; or (2) rotated in the vertical plane; or (3) kept stationary in the horizontal position and placed near to (1); or kept stationary in the vertical position and placed near to (2). After 5-6 days at 27°C, the polyps formed ephyrae in all of the groups, and the ephyrae were removed from the spines in the capsules and squashed in a wet film. The excess ASW was removed to flatten the animals, causing the statoliths to spread, so that the number of statoliths per rhopalium per ephyra could be counted and recorded (Figure 2). Statistical analyses were done on the number of statoliths formed per rhopalium using an ANOVA and the Duncan's New Multiple Range Test.

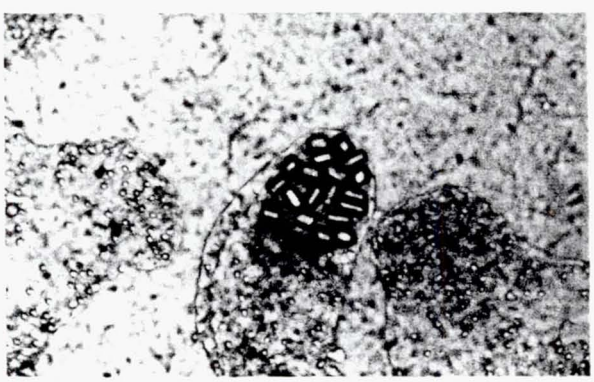

Figure 2. Statoliths spread in ephyra squash preparation.

RESULTS

Comparison of the numbers of statoliths formed by ephyrae which had developed during clinostat rotation in the horizontal plane with controls were made using an ANOVA which revealed significant differences between the groups at the $p<.01$ level and the Duncan's New Multiple Range Test. The latter test showed that those ephyrae which developed during rotation in the horizontal plane or 1/4 or 1/2 rpm had significantly fewer statoliths than controls which were rotated at these rates in the vertical plane or were kept stationary (Table 1). Ephyrae rotated in the horizontal plane at the other speeds of rotation did not have significantly different statolith numbers than controls.

Table 1. Numbers of statoliths in ephyrae which developed during clinostat rotation. (120 ephyrae in 3 tests per rotation speed).

Duncan's New Multiple Range Test

Rotation Speed (rpm)	Clinostat (Horizont. Plane)	Clinostat (Vertical Plane)	Control (Horizont. Plane)	Control (Vertical Plane)
1/15	25.7	25.6	26.3	23.8
1/8	22.0	19.9	19.2	17.0
1/4	*20.2	25.1	25.0	27.3
1/2	*22.7	25.4	25.5	24.5
1	22.0	16.9	20.9	17.4
24	32.7	27.6	30.5	29.6

*Mean numbers of statoliths per rhopalium of ephyrae rotated in the horizontal plane which are significantly different from controls. $p=.01$

DISCUSSION

A variety of developmental and maturational effects have been found in plants and animals following clinostat rotation in the horizontal plane (Miquel, 1984; Brown, 1984; and Walgemuth and Grills, 1984). Clinostat effects have been found using both slow and fast clinostat rotation. The reduced statolith formation in the jellyfish ephyrae rotated at 1/4 and 1/2 rpm is a response to slow clinostat rotation. Other biological systems responding to slow clinostat rotation in the horizontal plane include: the formation of more developmental anomalies in R. pipiens and X. laevis following rotation of their egg at 1/4 rpm (Tremor and Souza, 1972); disturbed tobacco protoplast regeneration by rotation at 4 rpm (Iversen and Baggerud, 1984); and the delay of flower formation, seed production, and seed maturation which was found by Hoshizaka (1984) following rotation of Arabidopsis following clinostat rotation at 1/4 rpm.

A feature common to clinostat studies is the finding (as in the jellyfish statolith research) that rotation of organisms at some clinostat speeds elicit biological responses whereas no response occurs following rotation at other speeds. Lyon (1979) while referring to his experiments with Coleus and tomato plants reported that the difference in rotational time requirements for organs with different patterns of growth and hormonal controls illustrates the impossibility of setting a fixed rule for optimal rate of rotation of a clinostat. Iverson and Baggerud (1984) were unable to decide from their experiments which type of clinostat (fast or slow) provides the best simulated microgravity environment. These authors concluded that the response of their organisms indicated them to be a sensitive tool for studies of gravity effects which should be further tested in a real microgravity environment. Gruener (1984) also concluded that "it is impossible at present to assess the fidelity with which clinostat rotation simulates zero hypogravity encountered in space". Brown (1984) compared biological responses of plants rotated on the clinostat with those exposed to the microgravity environment of space. He found that circumnutation in plants occurred more vigorously in space than on the earth-based clinostat.

The mechanisms through which clinostat-rotation in the horizontal plane reduces statolith numbers in developing ephyrae is not known. In other organisms, such rotation has been reported to: cause mixing of intracellular constituents in frog eggs (Tremor and Souza, 1972); reduce movement of an auxin in Coleus and tomato plants (1970); significantly alter the functional interactions between the elements of a prototypic synapse in cultured cells of X. laevis (Gruener, 1984); cause a decrease in the content of starch grains in chloroplasts in protoplasts of tobacco using the slow but not the fast clinostat and to produce minor differences between protein patterns of rotated protoplasts and controls (Iversen and Baggerud, 1984).

Statolith synthesis in rhopalia of Aurelia ephyrae appears to be sensitive to disorientation of the organisms with respect to gravity. It is, therefore, possible that statolith synthesis will also be affected by exposure of developing ephyrae to the microgravity of space. Indeed, changes occurring in rhopalia following development in space could provide information which can lead to the identification of mechanisms through which microgravity affects statolith synthesis and through which gravity influences normal development of statoliths and rhopalia on earth.

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